LA-UR--91-2100

DE91 014592

LAUR 91- 2100

TITLE: **PLANT GROWTH IS INFLUENCED BY GLUTAMINE SYNTHETASE-CATALYZED NITROGEN METABOLISM**

AUTHOR(S): **Pat J. Langston-Unkefer**

SUBMITTED TO: **Invited paper in Proceeedings for the DOE Workshop "Marker-Aided Selection", June 13 - 14, 1991. To be published in Tree Physiology.**

LosAlamos Los Alamos National Laboratory
Los Alamos, New Mexico 8754

Form No. 836 R4

MASTER

Summary

Ammonia assimilation has been implicated as participating in regulation of nitrogen fixation in free-living bacteria. In fact, these simple organisms utilize an integrated regulation of carbon and nitrogen metabolism; we expect to observe an integration of nitrogen and carbon fixation in plants; how could these complex systems grow efficiently and compete in the ecosystem without coordinating these two crucial activities? We have been investigating the role of ammonia assimilation in regulating the complex symbiotic nitrogen fixation of legumes. Just as is observed in the simple bacterial systems, perturbation of ammonia assimilation in legumes results in increased overall nitrogen fixation. The perturbed plants have increased growth and total nitrogen fixation capability. Because we have targeted the first enzyme in ammonia assimilation, glutamine synthetase, this provides a marker that could be used to assist selection or screening for increased biomass yield.

Plant Growth is Influenced by Glutamine Synthetase-Catalyzed Nitrogen Metabolism

Introduction

Biomass has been a valuable fuel for centuries and it will have considerable utility in the future because it can be converted to other fuels such as alcohol or methane, in addition to being used directly (Kitani and Hall 1989, National Research Council 1990, Oak Ridge National Laboratory 1989, OTA 1980, Venkatasubramanian and Keim 1985). In recent years, impressive technical advances have been made that increase the yield of biomass per acre (Department of Energy 1983, Oak Ridge National Laboratory 1987a, Oak Ridge National Laboratory 1987b), but further increases are needed to improve the cost effectiveness of biomass as an alternative energy source (Oak Ridge National Laboratory 1989). A major emphasis is improving the germplasm (National Research Council 1990) of the high biomass yielding plants. Another promising strategy for improving biomass production is the use of plant growth-promoting compounds. Both herbaceous and woody plants can be grown for biomass. Sudan grass, lespedeza, napier grass, switch grass, and various canes are among the most promising herbaceous plants. Alder, *Populus,* sycamore, sweetgum, and eucalyptus are among the promising trees.

The focus of our laboratory is to increase nitrogen fixation in legumes important for biomass or forage production. Legumes are an important part of an integrated biomass production program because they are high biomass-yielding plants that can grow well in areas not appropriate for non legumes or traditional food crops. Legumes are traditionally valuable as food stuffs and livestock feed because of their good yields and high nitrogen and protein contents. They have also been used world wide to release fixed nitrogen (ammonia) and thus build soils by fertilizing them. These beneficial properties of legumes make them extremely valuable to the world agricultural economy. Increasing legumes' biological nitrogen fixation is expected to improve their overall production, the cost-effectiveness of this production, their capacity to build soils, and their total nitrogen and protein contents. This increase in nitrogen fixation could also be expected to improve the biomass production of legumes.

Nitrogen fixation is the biological reduction of atmospheric nitrogen gas to ammonia, a form of nitrogen useful to plants and microorganisms. Several types of living organisms fix nitrogen; these include legume plants and certain free-living bacteria. Legumes fix nitrogen with the aid of a symbiotic relationship they form with specific bacteria, the Rhizobia and Bradyrhizobia; neither the plant nor these bacteria can fix nitrogen without the other partner (Beringer et al. 1979). Legumes allow these specific bacteria to invade their roots, and this invasion triggers the formation of specialized root structures, termed nodules. These nodules house the invading bacteria which in turn provide the nitrogen fixation machinery. The the legume provides fixed carbon (photosynthate) that feeds the nodule bacteria and supplies all of the energy needed to fix atmospheric nitrogen to ammonia (for excellent reviews and monographs see Haaker 1988, Postgate 1987, Ludden and Burris 1985, Gresshoff et al. 1991).

Because of the benefits mentioned above, we undertook work addressing the regulation of overall nitrogen fixation in legumes. Our technical approach was based upon several lines of evidence and basic biochemical principles. **1.** The plant must control the interaction in order for a legume to fix nitrogen and prosper. **2.** Extensive investigations of the regulation of simple free-living nitrogen fixing bacterial systems show that assimilated nitrogen regulates nitrogen fixation. **3.** First principles of biochemistry attribute control or regulatory properties to the first step in a metabolic pathway.

1. *The plant regulates the extent of the bacterial association; i.e., the plant regulates the formation of the root nodules where the bacteria are housed and where nitrogen is fixed.* In this way the plant tightly controls the bacterial growth and function in this beneficial symbiotic association. Thus, symbiotic associations are in sharp contrast to plant disease development in which deleterious effects follow an uncontrolled bacterial growth. These facts are also consistent with man's understanding of the evolution of legumes' ability to form symbiotic associations that fix nitrogen (Sprent and Sprent 1990).

2. *The amount of nitrogen an organism has assimilated regulates nitrogen fixation in simple systems.* Nitrogen fixation in the simple bacterial systems, i.e., those bacteria that fix nitrogen without associating with a legume, the free-livers, is regulated by the amount of fixed nitrogen they have. This nitrogen fixation system is

not regulated by the amount of ammonia that is available; but rather, it is regulated by the amount of fixed nitrogen (ammonia) they have assimilated into themselves. The first step in nitrogen assimilation, is the glutamine synthetase-catalyzed formation of glutamine from glutamate and ammonia. Glutamine contains and donates the nitrogen that the bacterium uses to make other amino acids, and ultimately all other nitrogen-containing compounds; thus the formation of glutamine is central in the cell's nitrogen metabolism. Thus, glutamine synthetase acts at an early and crucial point in this metabolism.

Research has begun to reveal how these bacteria detect and even measure the amount of nitrogen they are assimilating (selected references are in Table 1). The cells detect the ratio of available carbon, in the form of α-ketoglutarate, to assimilated nitrogen, in the form of glutamate or glutamine (Table 1). Thus, it is clear glutamine formation is a part of the measurement of the amount of assimilated nitrogen, and is coupled to regulating overall nitrogen fixation. Put simply, the bacteria detects how much nitrogen it has that is truly useful to it. It does not respond to the supply of nitrogen outside itself, it only responds to nitrogen it has assimilated. For this reason, ammonia, is not a biochemical regulator; only assimilated nitrogen is included in the measurement scheme. In this way, their nitrogen fixation is regulated by assimilated nitrogen. This global regulation system is termed the nitrogen regulation system, or *ntr*. The molecular genetic details of this system are being unraveled and many of the steps in the cascade of regulation machinery that begins with the detection and measurement of the α-ketoglutarate to glutamine ratio are now understood. This regulation system controls and integrates nitrogen metabolism with carbon availability; it controls glutamine synthetase activity, nitrogen fixation, and the cellular transport of dicarboxylic acids.

3. As early workers probed the routes and control of such basic metabolic pathways as glycolysis, the pentose pathway, and the Krebs cycle, several fundamental principles of biochemistry emerged. One of these first principles is that the first step in a metabolic pathway is likely to be a point of metabolic control or regulation. The first step in the pathway will usually be rate-limiting to allow for control of the rate of flow through the pathway. Thus, first steps often act as control points. It follows that the first step in a metabolic pathway is also a good place to measure the amount of flow through the pathway. In this case, glutamine synthetase activity is a good place to measure the amount of nitrogen being assimilated.

These three basic points can be restated and summarized. Because the plant controls the symbiotic association and because the plant assimilates the ammonia (produced by nitrogen fixation), then the first step in the plant's ammonia assimilation is expected to be involved in the regulation of the overall nitrogen fixation in legumes.

The approach outlined above can potentially identify a genetic marker for increased symbiotic nitrogen fixation and the accompanying increase in plant growth and biomass production. Quantitatively-inherited characters have been studied and associated with molecular markers in several crops (Tanksley et al. 1982, Osborn et al. 1987, Zamir et al. 1984, Tanksley and Iglesias-Olivas 1984, and Stuber et al. 1987, Edwards et al. 1987, Tanksley and Hewitt 1988, Martin et al. 1989, Paterson et al. 1988). These previous studies began with a specific quantitative trait and then associated the trait with a specific molecular marker; our study seeks to use biochemical and classical genetic approaches to identifying the molecular marker, which we have defined as a regulatory step.

Materials and Methods

Generation of soybean mutants

Our approach to obtaining true root glutamine synthetase-impaired mutants was modeled on the successful work of Dr. J. Harper and his coworkers (Nelson et al. 1983; Ryan et al. 1983). Our screen for impaired glutamine synthetase mutants was done in roots of young M2 seedlings, ultimately derived from chemically mutagenesis. We developed a quick assay to distinguish those seedlings whose roots contain significant versus significantly reduced amounts of glutamine synthetase activity. This first quick screen allowed us to reduce the number of plants that possibly contain glutamine synthetase mutations to manageable numbers. Our study of 12,000 plants detected 25-30 such candidate plants. These candidates were grown to maturity and seeds harvested from individual plants. We expected to find that most of the candidates were not the desired single gene mutation in glutamine synthetase, but rather were plants otherwise damaged by the mutagen. This was the case. As a consequence of this damage, they had generally suppressed metabolic activities and were actually false glutamine synthetase mutants. These false mutant plants were eliminated by growing

the mutant candidate plants to maturity and testing their progeny for the impaired-glutamine synthetase phenotype. At this point, we can be confident that the progeny that are reduced in glutamine synthetase activity are indeed true breeding for the reduction in glutamine synthetase activity. However, we will not yet know if these glutamine synthetase mutants are indeed the result of a single nuclear gene mutation. To make this determination, we will cross the mutants to wild-type plants and examine the segregation of the impaired-glutamine synthetase trait. The impaired-glutamine synthetase trait will segregate in simple Mendelian fashion in the F2 and F3 generations from an F1 cross with wild-type.

Results

As discussed above, to increase nitrogen fixation we targeted what appears to be a regulatory point, glutamine synthetase - catalyzed ammonia assimilation. We have employed two targeting strategies to selectively perturb glutamine synthetase - catalyzed ammonia assimilation in plant roots. 1.) Chemical inhibitors of glutamine synthetase have been tested. 2.) Specific mutation of the assimilatory glutamine synthetase in plant roots has been accomplished and is being fully tested.

Our target is the first step in ammonia assimilation in legumes, which is catalyzed by the plant enzyme, glutamine synthetase. Ammonia produced by nitrogen fixation is assimilated by the plant glutamine synthetases in root nodules of normal legumes. These root nodules contain two glutamine synthetases, one termed "root" glutamine synthetase and the other termed "nodule-specific" glutamine synthetase. ("Root" glutamine synthetase is a confusing, although common, term because "root" glutamine synthetase is present in both roots and nodules.) In our work this "root" glutamine synthetase in the roots and nodules is altered by one of two methods.

1. Selective targeting of glutamine synthetase forms with a chemical inhibitor: Treatment of legumes (alfalfa and soybeans) with a chemical selective for the "root" glutamine synthetase is accompanied by increased legume growth, increased nodule numbers, and increased nitrogen fixation (Tables 2 and 3) (Knight and Langston-Unkefer 1988a, Langston-Unkefer et al. 1991). This chemical is tabtoxinine-ß-lactam (TßL), which is produced by *Pseudomonas syringae* pv. *tabaci*. Treatment with TßL selectively impairs the "root" glutamine synthetase; TßL was either delivered to the

legume roots by *P. syringae* pv. *tabaci* (Table 2) (Knight and Langston-Unkefer 1988a, Langston-Unkefer et al. 1991) or the pure compound was applied directly to the root systems (Table 3). The effectiveness of the pure compound in duplicating the increased plant growth observed with the bacterially delivered TßL, eliminates any possible complications in interpretation of the data that could have been associated with the presence of *P. syringae* pv. *tabaci*.

2. Specific genetic targeting: One method of targeting a specific form of an enzyme is mutation breeding. This approach is most useful for obtaining mutants in plants that are relatively simple genetically, such as diploids or functional diploids. Developing this genetic approach provides the basic technology needed to genetically alter other important plants, such as some of the high biomass producing species.

Using classical mutation breeding in soybean we developed two glutamine synthetase mutants. These mutants are impaired in their "root" glutamine synthetase. These mutants were compared with wild type plants growing over the winter in a growth chamber; their significantly increased growth is shown in the accompanying table (Table 4). Complete characterization of these mutants will be needed to fully assess the impact of impairment of "root" glutamine synthetase activity. This characterization is underway and is a part of this new biomass effort.

Discussion

These results suggest that selective perturbation of ammonia assimilatory glutamine synthetase activity is beneficial to overall plant growth. And, if the mechanism regulating overall nitrogen fixation in free-living nitrogen fixing bacteria is generally conserved in legumes, then we expect to observe alteration in the total nitrogen fixation in plants with abnormal amounts of glutamine synthetase activity. Thus if plants have less than normal amounts of assimilatory glutamine synthetase activity, these plant roots and nodules will *apparently* have "insufficient assimilated nitrogen". The expected consequences of this message is to permit increased total nitrogen fixation capacity. Plants clearly regulate the extent of nodulation and this regulation is sensitive to the total amount of nitrogen being assimilated by glutamine synthetase. Thus, a straight-forward means of increasing total nitrogen fixation capacity is to permit a greater extent of nodulation; increased nodulation accompanied

selective impairment of alfalfa (Knight and Langston-Unkefer 1988a) and soybean (Langston-Unkefer et al. 1991) "root" glutamine synthetase. Another means of increasing total nitrogen fixation capacity is to increase the amount of fixation capacity per unit weight of nodule; such an increase was observed in alfalfa (Knight and Langston-Unkefer 1988a) and soybean (Langston-Unkefer et al. 1991) nodules. These results indicate that glutamine synthetase activity is kinetically limiting and thus changes in glutamine synthetase activity are observed to effect the concentrations of glutamine in the nodule (Knight and Langston-Unkefer 1988)a. This is another important characteristic of an enzyme whose activity is important in a biological information transduction scheme.

Our findings indicate that the amount of "root" glutamine synthetase activity is important in regulating or specifying total nitrogen fixation and ultimately plant growth. Thus, our findings suggest that glutamine synthetase activity could be a valuable biochemical marker for increased performance. Plants with reduced levels of glutamine synthetase could either be screened from in natural populations or mutants with reduced amounts of root glutamine synthase could be created and used in breeding schemes.

In addition to our findings with plants, the results in other laboratories are consistent with ammonia assimilation regulating total symbiotic nitrogen. Workers at Minnesota examined a natural population of alfalfa plants and divided these plants into two groups of plants: those with high or low nodule nitrogen fixation activity. Examination of these two groups of plants revealed that the high nitrogen fixing plants had a lower amount of ammonia assimilatory activity (glutamine synthetase - glutamate synthase) relative to their nodule carbon fixation capacity (PEP carboxylase) (Jessen et al. 1987). Thus, these plants provide additional evidence that the ratio of assimilated nitrogen to carbon is important in regulating overall nitrogen fixation. The altered ratio of enzyme activities they observed could readily alter the ratio of α-ketoglutarate to glutamine in the nodules.

These findings provide one with several alternatives to further exploration and development of this control of the natural regulation system working in legumes. These approaches are treatment with selective chemical inhibitors of glutamine synthetase activity, generation of specific glutamine synthetase null mutants, and a third approach that we have not yet employed, which is the selection of populations of

plants with reduced amounts of root glutamine synthetase activity. Trees could be treated with selective inhibitors of glutamine synthetase activity; although this approach requires continuous treatment, it could be effective in enhancing seedling growth and thus help to reduce time to harvest. Generation of mutants in trees is a lengthy process because most trees have long generation times. This third option, selection of trees with reduced levels of root glutamine synthetase activity, is an attractive technical approach to testing and developing the approach in tree species with important biomass production capability.

We expect that the implementation of this approach will require considerable understanding and development. A number of factors will need to be considered and some of them will be specific to trees. These include understanding the effect of impairment or complete inactivation of the root glutamine synthetase activity on high biomass-yielding trees. Trees normally assimilate much of their nitrogen in their leaves and thus, impairment of their root assimilatory activity may not perturb trees to the same degree as a plant that normally assimilates more of its nitrogen in its roots. We have found that oats, which have about 60% of their glutamine synthetase in their leaves and 40% in their roots, have faster growth, and increased leaf protein and glutamine synthetase activity when their root glutamine synthetase is inactivated (Knight and Langston-Unkefer 1988b). We can not predict the extent of the increased growth in these plants. Another unknown is the role of root nodule nitrogen assimilation and the establishment of the symbiotic association between the tree and its symbiont; will nitrogen-fixing trees respond to glutamine synthetase impairment as the legumes respond? Another factor to be considered will be effects of genetic background, including varietal or cultivar differences.

Other factors are more general and probably apply to all nitrogen fixing plants. The initial findings stimulate an important basic question: How can plants support increased nitrogen fixation? Are they photosynthate limited or can they be induced to fixed more carbon if more nitrogen is available? It seems completely reasonable that plants can fix more carbon because only a fraction of their carbon fixation enzyme, ribulose bisphosphate carboxylase, is active in plant tissues; this carbon fixing enzyme is activated by another enzyme, indicating that its activity is regulated in a precise manner. The biochemical rationale or fundamental principles underlying this regulation are not yet known. Why should a plant limit its carbon fixation? One situation exists when insufficient nitrogen is available to allow the plant to use the fixed

carbon. Plants use fixed carbon and assimilated nitrogen (and phosphorus etc) to synthesize all of their cellular components in prescribed ratios of carbon to nitrogen content. This simple observation indicates that carbon and nitrogen metabolism must be closely linked and coordinated; such coordination is crucial in plants where carbon and nitrogen are obtained by separate processes.

To begin to address these considerations, our investigation of this system will include a careful characterization of the "root" glutamine synthetase-impaired mutant soybeans. The characterization includes biochemical, genetic, and field testing. We will also extend our investigations of the amino acid analogs that stimulate plant growth.

References

Arp, D.J. and W.G. Zumft 1983 L-Methionine-SR-sulfoximine as a probe for the role of glutamine synthetase in nitrogenase switch-off by ammonia and glutamine in *Rhodopseudomonas palustris.* Arch. Microbiol. 134: 17-22.

Ausubel, F.M. 1984 Regulation of nitrogen fixation genes. Cell 37: 5-6.

Beringer, J. E., N. J. Brown, A. W. B. Johnston, H. M. Schluman and D. A. Hopwood 1979 The Rhizobium -legume symbiosis. Proc. R. Soc. Lond. B204: 219-233.

Cejudo, F.J., A. de la Torre and A. Paneque 1984 Short-term ammonium inhibition of nitrogen fixation in *Azotobacter.* Biochem. Biophys. Res. Comm. 123: 431-437.

Department of Energy, Biomass Energy Technology Research Program Summary, FY 1983, DOE/CE-0032/1.

Edwards, M. D., C. W. Stuber, and J. F. Wendel 1987 Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution, and types of gene action. Genetics 116: 113-125.

Gibson, A.H. and J.E. Harper 1985 Nitrate effect on nodulation of soybean by *Bradyrhizobium japonicum.* Crop Science 25: 497-501.

Gresshoff, P. M., L. E. Roth, G. Stacey, and W. E. Newton 1991 Nitrogen Fixation: Achievements and objectives. Chapman and Hall, New York.

Gussin, G.N., C.W. Ronson, and F.M. Ausubel 1986 Regulation of nitrogen fixation genes. Ann. Rev. Genet. 20: 567-591.

Haaker, H. 1988 Biochemistry and physiology of nitrogen fixation. BioEssays 9: 112-117.

Hoechst Patent, # HOE 82F 002, Issuing country, Germany.

Imsande, J. 1986 Inhibition of nodule development in soybean by nitrate or reduced nitrogen. J. Exptl. Bot. 37: 348-355.

Jessen, D.L., D.K. Barnes, C.P. Vance and G.H. Heichel 1987 Variation for activity of nodule nitrogen and carbon assimilating enzymes in alfalfa. Crop Sci. 27: 627-631.

Jones, B.L. and K.J. Monty 1979 Glutamine as a feedback inhibitor of the *Rhodopseudomonas sphaeroides* nitrogenase system. J. Bacteriol. 139: 1007-1013.

Kitani and Hall, Eds., 1989 Biomass Handbook, Gordon and Brad Publishers.

Knight, T. J. and P. J. Langston-Unkefer 1988a Enhancement of symbiotic dinitrogen fixation by a toxin-releasing plant pathogen. Science 241: 951-954.

Knight, T. J., D. R. Bush, and P. J. Langston-Unkefer 1988b Oats tolerant of Pseudomonas syringae pv. tabaci contain tabtoxinine-ß-lactam-insensitive leaf glutamine synthetases. Plant Physiol. 88: 333-339.

Knight, T.J., R. D. Durbin, and P. J. Langston-Unkefer 1986 Effects of tabtoxinine- ß-lactam on nitrogen metabolism in *Avena sativa* L. roots. Plant Physiol. 82: 1045-1050.

Langston-Unkefer, P. J., T. J. Knight, and C. Sengupta-Gopalan 1991 Beneficial consequences of a selective glutamine synthetase inhibitor in oats and legumes. Proceedings of International Symposium and Workshop on Biological Nitrogen Fixation Associated with Rice Production. Oxford India Publishing.

Ludden, P. W. and R. H. Burris 1985 Nitrogen fixation and CO_2 metabolism. Esleveir, New York.

Magasanik, B. 1982 Genetic control of nitrogen assimilation in bacteria. Ann. Rev. Genetics 16: 135-168.

Magasanik, B. 1988 Reversible phosphorylation of an enhancer binding protein regulates the transcription of bacterial nitrogen utilization genes. TIBS 13: 475-479.

Martin, B., J. Nienhuis, G. King, and A. Schaefer 1989 Restriction fragment length polymorphisms associated with water use efficiency in tomato. Science 243: 1725-1728.

Munns, D.N. 1977 Mineral nutrition and the legume symbiosis. *In* A Treatise on dinitrogen fixation. IV Agronomy and ecology. R.W.F. Hardy & A.H. Gibson, Eds. John Wiley & Sons, New York. p. 353-391.

National Research Council, Committee on Alternative Energy R & D Strategies Report, 1990. National Academy Press, Washington, DC.

Nelson, R. S., S. A. Ryan, and J. E. Harper 1983 Soybean mutants lacking constitutive nitrate reductase activity I. Selection and initial plant characterization. Plant Physiol. 72: 503-509.

Oak Ridge National Laboratory, Short Rotation Woody Crops Program: Annual Progress Report for 1987a. Environmental Sciences Division Publication #3030.

Oak Ridge National Laboratory. 1987b. Herbaceous Energy Crops Program: Annual Progress Report for 1987. Environmental Sciences Division Publication # 3191.

Oak Ridge National Laboratory. 1989. Energy Technology R&D. What Could Make a Difference?

Osborn, T. C., D. C. Alexander, and J. F. Foves 1987 Identification of restriction fragment length polymorphisms linked to genes controlling soluble solids content in tomato fruit. Theor. Appl. Genet. 73: 350-356.

OTA 1980. Energy from biological processes. Office of Technology Assessment. Washington, DC.

Paterson, A. H., E. S. Lander, J. D. Hewitt S. Paterson, S. E. Lincoln and S. D. Tanksley 1988 Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335: 721-726.

Postgate, J. 1987 Nitrogen fixation. Chapman and Hall, New York.

Reitzer, L.J. and B. Magasanik 1987 Ammonia assimilation and the biosynthesis of glutamine, glutamate, aspartate, asparagine, L-alanine and D-alanine. *In E. coli* and *Salmonella typhimurium:* Cellular and molecular biology. Ed. F.C. Neidhardt. American Society for Microbiology, pp 302-320.

Ryan, S. A., R. S. Nelson, adn J. E. Harper 1983 Soybean mutants lacking constitutive nitrate reductase activity II. Nitrogen assimilation, chlorate resistance, and inheritance. Plant Physiol. 72: 510-514.

Shanmugum, K.T. and C. Morandi 1976. Amino acids as repressors of nitrogenase biosynthesis in *Klebsiella pneumoniae.* Biochim. Biophys. Acta 437: 322-332.

Sprent, J. I. and P. Sprent 1990 Nitrogen Fixing Organisms: Pure and Applied Aspects. Chapman and Hall, London. pp 189-209.

Stuber, C. W., M. D. Edwards, and J. F. Wendel 1987 Molecular-marker-facilitated investigations of quantitative-trait loci in maize. II. Factors influencing yield and its component traits. Crop Sci. 27: 639-648.

Tanksley, S. D., H. Medina-Filho, and C. M. Rick 1982 Use of naturally-occurring enzyme variation to detect and map genes controlling quantitative traits in an interspecific backcross of tomato. Heredity 49: 11-25.

Tanksley, S. D. and J. Iglesias-Olivas 1984 Inheritance and transfer of multiple-flower character from *Capsicum chinense* into *Capsicum annuum.* Euphytica 33: 769-777.

Tanksley, S. D. and J. Hewitt 1988 Use of molecular markers in breeding for soluble solids content in tomato-a re-examination. Theor. Appl. Genet. 75: 811-823.

Tubb, R.S. 1976. Regulation of nitrogen fixation in *Rhizobium* sp. Appl. Environ. Microbiol. 32: 483-488.

Venkatasubramanian, K., and C. Keim 1985. Starch and Energy: Technology and Economics of Fuel Alcohol Production, *In* Starch Conversion Technology, Eds. Van Beynum , G. M. A., and J. A. Roels, Marcel Dekker, Inc., New York.

Yoch, D.C. and R.M. Pengra 1966. Effect of amino acids on the nitrogenase system of *Klebsiella pneumoniae.* J. Bacteriol. 92: 618-622.

Zamir, D., T. Selilaben-Davis, J. Rudich, and J. A. Juvick 1984 Frequency distribution and linkage relationships of 2-tridecanone in interspecific segregating generations of tomato. Euphytica 33: 481-488.

Table 1. Nitrogen Metabolism Regulates Nitrogen Fixation

System	Observed Effect	Control Agent	Ref's
Bacterial			
N regulatory system, ntr	Regulates gene expression for: a. N assimilation enzymes, b. Nitrogenase, c. C4 dicarboxylic transport	α-Ketoglutarate : Glu or Gln ratio	Ausubel, 1984 Gussin *et al.*, 1986 Magasanik, 1982 & 1988 Reitzer & Magasanik, 1987
Exogenous NH_3 inhibits nitrogenase	Nitrogenase activity regulated by covalent modification	Overcome by inactivating GS	Arp & Zumft, 1983 Cejudo, *et al.* 1984 Jones & Monty, 1979
Amino acids	Influence N_2 fixation	Not known	Tubb, 1976 Shanmugam & Morandi, 1976 Yoch & Pengra, 1966
Plant			
Root-nodule GS-impaired legumes	Greater plant growth, nodulation, N_2-fixation	GS function	Knight & Langston Unkefer, 1988 Langston-Unkefer, *et al.*, 1989
Exogenous N	Inhibits nodulation	At least 2 mechanisms	Imsande, 1986 Munns, 1977 Gibson & Harper, 1985
Root-nodule GS-unpaired legumes	NO_3 inhibition of nodulation partially overcome.	GS function	Knight & Langston-Unkefer, 1988
Nodule CO_2 fixation / N assimilation altered	Increased N_2 fixation	PEPC : GOGAT ratio of activities	Jessen *et al.*, 1987

Table 2. ***Effects of Treatment with Microbially Delivered TßL***

Treatment	Plant FWt (mg/plant)	Nodules / plant	Nitrogenase Activity U/mg protein	Root GS Activity U/mg protein
Alfalfa				
Control	121 (100%)	3.2	13.4	0.95
Treated	240 (199%)	7.1	19.8	0.06
Soybean				
Control	537 (100%)	75	7.4	2.5
Treated	690 (128%)	138	8.4	0.4

The root systems of nodulating legume plants were inoculated with *Pseudomonas syringae* pv. *tabaci* at 14 days after planting. Plants were grown in a growth chamber without nitrogen supplementation. The alfalfa plants were harvested at 30 days and the soybeans were harvested at 35 days. The full details were published perviously (Knight and Langston-Unkefer 1988, Langston-Unkefer et al. 1991).

Table 3. ***Effects of Treatment with Pure Inhibitors***

	Treatment	Nodules / Plant	Whole Plant Fresh Weight (% control)
Alfalfa*			
	Control (0 TßL)	7	100
	0.1 µmole TßL	12	216
Soybean **			Foliar Dry Weight
	Control	Not given	100%
	Phosphinothricin	Not given	~34%

* Nodulating alfalfa plants were treated with pure (by HPLC and amino acid analysis) TßL with the amounts given above beginning at 14 days after planting. TßL was applied to the root systems every three days for 21 days. The soil volume was 500 ml. Nodulated alfalfa was grown without nitrogen as described perviously (Knight and Langston-Unkefer 1988). Data were collected at the end of the treatment. 50 seedlings were used for each sample. ** Few details were provided in the Hoechst patent.

Table 4. ***Soybean "Root" Glutamine Synthetase Mutants***

Soybean Type	Above Ground* Fresh Wt gm/plant	Root GS** Activity Units/ mg protein
Wild type	246	2.5 (100%)
Mutant	339	1.1 (44%)

* Three mutant plants (50 days old) were sampled for these measurements. The mutants exceed the growth of the wild types by the same percentage at 30 days of age.

** Root GS activity was measured by using the same type of root tissue from the same part of the root system of each plant. 45 day old plants were used. All plants were grown in a growth chamber, with 16 h day length until flowering began and then changed to 11h day length. They were supplied with 10 mM nitrate and additional mineral supplementation.